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MÀSTER UNIVERSITARI EN OPTOMETRIA I CIÈNCIES DE LA VISIÓ

TREBALL FINAL DE MÀSTER

VISUAL AND MORPHOLOGICAL CHARACTERISTICS IN A PRE-TREATED EXUDATIVE AGE-RELATED MACULAR DEGENERATION POPULATION

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Terrassa, 22 de juny de 2017

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Les Sres. Sara Lluch Margarit i María Dolores Merindano Encina, com a directores del treball

CERTIFIQUEN

Que la Sra. Antía Suárez Vidal ha realitzat sota la seva supervisió el treball VISUAL AND MORPHOLOGICAL CHARACTERISTICS IN A PRE-TREATED EXUDATIVE AGE-RELATED MACULAR DEGENERATION POPULATION que es recull en aquesta memòria per optar al títol de màster en optometria i ciències de la visió.

I per a què consti, signem aquest certificat.

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CARACTERÍSTICAS VISUALES Y MORFOLÓGICAS EN UNA POBLACIÓN CON DEGENERACIÓN MACULAR ASOCIADA A LA EDAD EXUDATIVA PRE-TRATADA

RESUMEN

La degeneración macular asociada a la edad (DMAE) es la principal causa de ceguera legal en países desarrollados. La DMAE exudativa, en la cual se centra este estudio, cursa con neovascularización coroidea originando una importante disminución en la capacidad visual.

El objetivo de este estudio era determinar la influencia de la actividad patológica (presencia de fluido retiniano) y del número total de inyecciones en la eficacia del tratamiento, en una población previamente tratada con agentes anti-VEGF.

Para ello, 33 pacientes con DMAE exudativa fueron evaluados. Se estudió la calidad visual mediante las pruebas de agudeza visual, sensibilidad al contraste y visión del color, y la integridad neuroretiniana mediante OCT. Dicha evaluación se realizó en dos visitas (PRE y POST), con una inyección anti-VEGF de diferencia entre ambas.

Los resultados PRE y POST mostraron diferencias significativas para grosor retiniano y actividad patológica. Las retinas sin actividad previa presentaron valores más moderados en el resto de las variables. Sin embargo, el tratamiento pareció ser más efectivo en pacientes con PRE-actividad, ya que la reabsorción del fluido retiniano y la consecuente disminución del grosor favorecen la mejora en la calidad visual. En cuanto al número total de inyecciones, los resultados determinaron que mayor número de inyecciones no comportaba mejoras significativas.

Como conclusión, el tratamiento para DMAE exudativa en pacientes con actividad patológica parece ser más eficaz que en aquellos sin fluido retiniano. Además, el tratamiento mantiene la calidad visual y evita la progresión de la patología más que recuperar la funcionalidad visual.

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CARACTERISTIQUES VISUALS I MORFOLÒGIQUES EN UNA POBLACIÓ AMB DEGENERACIÓ MACULAR ASSOCIADA A L´EDAT EXUDATIVA PRE-TRACTADA

RESUM

La degeneració macular associada a l'edat (DMAE) és la principal causa de ceguesa legal en països desenvolupats. La DMAE exudativa, en la qual se centra aquest estudi, cursa amb neovascularització coroïdal originant una important disminució en la capacitat visual.

L'objectiu d'aquest estudi va ser determinar la influència de l'activitat patològica (presència de líquid retinal) i del nombre total d'injeccions en l'eficàcia del tractament en una població prèviament tractada amb agents anti-VEGF.

Així, 33 pacients amb DMAE exudativa van ser avaluats. Es va estudiar la qualitat visual mitjançant les proves d´agudesa visual, sensibilitat al contrast i visió del color, i la integritat neuroretiniana mitjançant OCT. Aquesta avaluació es va realitzar en dues visites (PRE i POST), amb una injecció anti-VEGF de diferència entre ambdues.

Els resultats PRE i POST van mostrar diferències significatives per al gruix retinal i activitat patològica. Les retines sense activitat prèvia van presentar valors més moderats a la resta de les variables. No obstant, el tractament semblava ser més efectiu en pacients amb PRE-activitat, ja que la reabsorció del fluid retinal i la conseqüent disminució del gruix afavoreixen la millora en la qualitat visual. Pel que fa al nombre total d'injeccions, els resultats van determinar que un major nombre d'injeccions no comportava millores significatives.

Com a conclusió, el tractament per DMAE exudativa en pacients amb activitat patològica sembla ser més eficaç que en aquells sense fluid retinal. A més, el tractament manté la qualitat visual i evita la progressió de la patologia més que recuperar la funcionalitat visual.

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VISUAL AND MORPHOLOGICAL CHARACTERISTICS IN A PRE-TREATED EXUDATIVE AGE-RELATED MACULAR DEGENERATION POPULATION

ABSTRACT

Age-related macular degeneration (AMD) is the leading cause of legal blindness in developed countries. Exudative AMD, in which present study is focused, presents with choroidal neovascularization inducing to an important decrease in visual capacity.

The aim of this study was to determine the influence of disease activity (presence of retinal fluid) and the total number of injections on the treatment efficacy in a pre-treated population with anti-VEGF injections.

For this, 33 patients with exudative AMD were evaluated. Visual quality was assessed by visual acuity, contrast sensitivity and colour vision tests, and neuroretinal integrity by OCT. This evaluation was performed in two clinical visits (PRE and POST), with an anti-VEGF injection of difference between both.

PRE and POST results showed significant differences for retinal thickness and disease activity. Non-active retinas displayed more moderate values in the rest of the variables. However, treatment seemed to be more effective in patients with PRE-activity, since the retinal fluid reabsorption and the consequent decrease in the retinal thicknesses favoured the visual quality improvement. Regarding the number of total injections, the results determined that more injections did not achieve a significant improvement.

In conclusion, the treatment for exudative AMD in patients with disease activity seems to be more efficient than in those without retinal fluid. In addition, treatment maintains visual quality and avoids the progression of pathology rather than visual function recovery.

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COVER LETTER

Dear Editor,

Attached you will find the paper entitled “Visual and morphological characteristics in a pre-treated exudative Age-Related Macular Degeneration population”, which we are submitting for publication in *Retina* as an “Original Research paper”.

To determine whether disease activity pattern and total number of anti-VEGF injections are determining in the effectiveness of exudative AMD treatment, visual function and morphological retinal integrity were evaluated. Neuroretinal integrity and disease activity were analysed by OCT imaging as well as visual function was evaluated by visual acuity, contrast sensitivity and colour vision tests in 33 patients with exudative AMD. Results showed that neither visual nor morphological changes were significant, only substantial improvements were found in resolution of oedema and thus reduction of retinal thickness. Furthermore, no statistically differences were found according to number of previous injections, as treatment in exudative AMD appeared to maintain visual abilities.

We state that the data submitted have not been published and are not under current considerations elsewhere, that all authors have contributed significantly in the design, interpretation of data and all are in agreement with the content of the study.

We would be very grateful for any comments or suggestions you may wish to make.

Thanking you for your kind attention, we look forward to hearing from you at your convenience.

Yours sincerely,

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TITLE PAGE

Title: Visual and morphological characteristics in a pre-treated exudative Age-Retaled Macular Degeneration population.

Abbreviated title: Outcomes of the exudative-AMD treatment

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KEY WORDS AND SUMMARY STATEMENT

Key Words: Age-related macular degeneration; Anti-VEGF; Colour vision; Contrast sensitivity; Choroidal neovascularization; Exudative AMD; Optical Coherence Tomography; Retinal oedema; Retinal thickness; Visual acuity

Summary Statement: Visual and morphological traits were evaluated in patients with exudative Age-Related Macular Degeneration pre-treated with anti-VEGF injections to determine the influence of disease activity and the total number of injections in the efficiency of the treatment.

ABSTRACT

Purpose – To evaluate the visual and morphological variables of a pre-treated exudative AMD population, determining the influence of the activity pattern and the total number of anti-VEGF injections in the results.

Methods – Thirty-three patients with exudative AMD were evaluated before and one month after anti-VEGF injection treatment. Visual acuity, contrast sensitivity and colour vision were analysed to assess visual function. Retinal thickness, neuroretinal integrity and disease activity pattern were evaluated by OCT imaging. Study was divided in: comparison PRE versus POST outcomes; comparison of the results according to previous disease activity pattern; comparison of the results according to the number of previous injections.

Results– One additional anti-VEGF injection only involved statistical changes in retinal thicknesses and pathology activity. Non-active PRE retinas showed moderate values for the studied variables, although treatment was more effective in active PRE retinas with retinal oedema. No statistical differences were found in the results according to the number of total injections.

Conclusions – The resolution of oedema and, thus the reduction of retinal thicknesses, seem to be the only substantial improvement that anti-VEGF treatment provides, since neither visual nor morphological changes are statistically significant. In addition, treatment in exudative AMD appeared to maintain visual abilities and prevent disease progression

INTRODUCTION

Age-related macular degeneration (AMD) is the leading cause of legal blindness among the elderly people in developed countries, accounting for 8.7% worldwide.[1, 2, 3] In Spain, the prevalence of AMD is 3.4%, corresponding 1.5% to dry form (also known as atrophic, which includes drusen and geographic atrophy of the retinal pigment epithelium), and 1.9% to wet or exudative form which is the more aggressive form of the pathology.[2]

Exudative AMD is mainly characterized by choroidal neovascularization.[4] It is thought that hypoxia is the most important factor involved in this neovascularization, causing abnormal growth of fragile blood vessels. Furthermore, a stimulation of angiogenesis by activating transcription of the gene VEGF (Vascular endothelial grow factor) has been demonstrated both in vitro and in vivo. These facts produce dysfunction of the blood-retinal barrier, resulting in leakages into the retinal tissues.[1] Consequently, the most frequent signs of exudative AMD are due to the choroidal neovascularization leakage that causes haemorrhages, hard exudates, macular serous elevation and retinal oedemas.[4]

In fact, the exudative AMD activity is ultimately based upon the presence of macular oedema. In this sense, sub-retinal or/and intra-retinal fluids, observed by optical coherence tomography (OCT) as dark empty spaces, are decisive not only to determine the disease activity, but also the treatment and follow-up protocols.[5, 6]

In some cases, the onset of exudative AMD can be asymptomatic, especially if the visual loss occurs in the non-dominant eye.[7] Most important symptoms are loss of central visual, metamorphopsia (visual distortion in which straight lines appear crooked), problems on dark adaptation, inability to distinguish fine detail and central blurring.[1, 7] Moreover, 46.5% of patients with AMD present a visual acuity <20/400 [2] and the prevalence of severe visual loss increases with age.[8] Risk factors include age, white race, sex (women), tobacco, cataracts and family background.

The impact of AMD on emotional well-being, activities of daily living and quality of life can be profound and appears to be directly related to the severity of the pathology. Because of it, early detection and prevention of progression are the most important public health goals.[9] Available

therapies are not causal treatments but generally, they avoid further vision loss rather than to improve vision. Anti-angiogenic agents attempt to block various steps in the pathway of angiogenesis in choroidal neovascularization.[3]

Three mainly types of treatment regimen are used in exudative AMD. Monthly injections with anti-VEGF agents, independently of the disease activity, have good results although many clinic visits are required. In *pro re nata* (PRN) regimen, the patients are usually given a loading dose of 3 monthly injections and then one anti-VEGF injection every 4 weeks, only in case of active disease. In *treat-and-extend* (TAE) regimen, after 3 monthly anti-VEGF injections, the intervals between treatments increase progressively, 2 weeks at a time, as long as the disease remains non-active, that is without no signs of oedemas.[5, 10-13]

To our knowledge, there are no studies relating the visual and morphological characteristics of exudative AMD patients according to the disease activity and the extension of the treatment received.

The aim of the present study was to examine the morphological and visual variables of a pre-treated exudative AMD population, evaluating the influence of the activity pattern and the number of anti-VEGF injections in the results.

METHODS

Subjects

Thirty-three patients, previously treated for exudative age-related macular degeneration (AMD) with intravitreal injections of anti-VEGF agents, were recruited from Hospital de Terrassa-Consorci Sanitari de Terrassa (Barcelona, Spain). Inclusion criteria were: patients aged > 55 years with diagnosed exudative AMD and visual acuity (VA) of at least 49 letters on the ETDRS chart. Exclusion criteria were: presence of another ocular pathology which could alter contrast sensitivity, colour vision or visual acuity (cataract, glaucoma, high myopia, and previous retinal surgery); some cognitive disease like Alzheimer or Parkinson; and being under some treatment which could alter colour vision. All the tests were carried out only in the eye diagnosed of AMD and, in case both eyes were affected, the eye with best VA was selected. All subjects provided

written informed consent to participate in this study, which has been approved by the ethical committee of the Terrassa Health Consortium and followed the tenets of the Declaration of Helsinki.

Procedures

Visual function tests were performed with best corrected refractive error previously established by autorefraction and subjective refraction. Visual acuity was examined with Early Treatment Retinopathy Study (ETDRS) chart, which consisted of 5 letters per line in 0.1 LogMAR steps. The distance between participants and ETDRS chart was 4 meters and test was done under mesopic conditions. If participants could see at least 20 letters at a distance of 4 meters, 30 letters were added to calculate the total number score; if not, chart was then situated at 1 meter and the total number of letters seen was the final score. In this way, more precise results are obtained since it indicates the exact number of letters instead of the number of lines seen by the patients, as other optotypes do. Higher letter scores correspond to better VA. The total numbers of letters were then translated into LogMAR notation for statistical analysis.[14, 15]

Contrast sensitivity (CS) was measured with Pelli-Robson chart, comprising 8 lines with 2 triplets (three letters) per line, 16 triplets in total. Each triplet has different contrast, which decreases line to line from 100% to 0.6%, in units of 0.15 log. Distance between patients and chart was about 1 meter, which corresponds to a spatial frequency of 1 cycle per degree. Contrast sensitivity was measured under photopic conditions (under bright-ambient room illumination). Test final score corresponds to the triplet in which at least 2 letters are correctly identified. The results were then converted into logarithmic contrast sensitivity (logCS) notation for statistical analysis. Mean normal value in healthy patients aged > 60 is 1.71 logCS. [16, 17]

Farnsworth Dichotomous test or D-15 panel was used to examine the colour vision deficiency. This test consists in 15 caps of different colours, which participants must order according to their chromatic similarity. It was performed with a near optic correction and under photopic conditions. Results were reproduced in a diagrammatic representation in which the colour vision defect type and severity can be observed.[18] Furthermore, Total Colour Difference Score (TCDS) and Confusion Index (C-index), were calculated as colour vision indexes. TCDS is a quantitative

indication of the cap transpositions, with an ideal mean value of 125.1 for the age group over 60 years; [18] CI estimates the severity of the color vision alteration, ranging from 1 up to 4 for strong vision deficiencies. [19]

Central retina imaging was performed with Optovue OCT (Avanti, Optovue, USA; www.optovue.com), a system that uses 840nm wavelength light and provides 70000 A-scan/second, with an optical resolution of 5µm (axial) and 15µm (transversal). The obtained images were used to measure central retinal (CRT) and perifoveal thicknesses (PFT), considering the mean values of 256µm and 284µm respectively [20] and to study some morphological traits related with exudative AMD. In that sense, the activity pattern of the pathology (non-active and active) according to the absence or the presence of retinal fluid (RF), the integrity of the ellipsoid zone (EZ, previously referred as IS/OS), [21] the external limiting membrane (ELM) for the outer retinal layers, and the disorganization of the retinal inner layers (DRIL) [22] were evaluated.

All tests were realized twice, before the patients were treated by an anti-VEGF intravitreal injection and one month after the treatment. Thus, results were grouped in before and after-treatment outcomes (PRE and POST groups, respectively**)**

According to the aims anteriorly described, this study was divided in three phases: comparison between the PRE and POST outcomes; comparison of these two groups depending on the activity pattern prior to treatment; and comparison of the POST outcomes in function of the number of injections received before to the current study.

Statistical Analysis

Statistical analysis was performed using SPSS software V23.0 (SPSS, Chicago, IL, USA). After a preliminary exploratory analysis, the quantitative variables were examined for normality using the Kolmogorov–Smirnov test/Shapiro-Wilk test, and Mann-Whitney U test or Wilcoxon signed-rank test were carried out for comparisons between groups. For categorical variables, intergroup differences were stablished by Chi-squared (or Fisher exact test when $n<5$) and McNemar tests. A p value of 0.05 or less was considered to denote statistical significance throughout the study.

RESULTS

Thirty-three patients, 16 women (48.49%) and 17 men (51.52%), aged between 64 to 92 years (mean=80.78, SD=6.80) underwent the PRE-treatment testing, while only twenty-eight patients could be evaluated in the POST-treatment tests, because 5 patients did not return for the second evaluation.

1. Comparison between PRE and POST outcomes

Summary statistics and the frequency distribution of visual and morphological variables for both PRE and POST groups are shown in table 1, 2 and 3**.**

	PRE (n=33)				POST (n=28)			
	LogMAR	**LogCS**	**TCDS**	**CI**	**LogMAR**	**LogCS**	**TCDS**	**CI**
Mean	0.31	1.29	231.77	2.54	0.27	1.30	214.85	2.35
Median	0.30	1.35	236.30	2.70	0.24	1.35	221.80	2.36
SD	0.20	0.19	58.97	0.63	0.25	0.20	57.69	0.72
Min	0.00	1.05	122.70	1.13	-0.8	0.90	117.00	1.00
Max	0.70	1.65	406.80	3.54	1.10	1.62	341.90	3.67

Table 1. Descriptive statistics of visual variables for both PRE and POST groups. CI: confusion index; LogCS: contrast sensitivity logarithm; LogMAR: Logarithm of the Minimum Angle of Resolution; TCDS: Total Colour Difference Score**.**

	PRE (n=33)		POST (n=28)	
	CRT	**PFT**	**CRT**	**PFT**
Mean	274.12	270.70	240.65	255.92
Median	263.00	261.00	242.00	255.00
SD	63.56	35.95	29.71	25.71
Min	198.00	198.00	165.00	207.00
Max	448.00	362.00	309.00	320.00

Table 2. Descriptive statistics of retinal thickness for both PRE and POST groups. CRT: central retinal thickness; PFT: Perifoveal thickness, both expressed in µm

		PRE n=33	POST n=28
		%	%
ACTIVITY	**Non-active**	42.4	85.2
	Active	57.6	14.8
RF	**Intraretinal**	31.6	0.0
	Subretinal	47.4	100.0
	Intra + sub	21.0	0.0
EZ	**Normal**	45.5	70.4
	Altered	54.5	29.6
ELM	**Normal**	72.7	85.2
	Altered	27.3	14.8
DRIL	**Absence**	81.8	88.9
	Presence	18.2	11.1

Table 3. Frequency distribution of qualitative OCT morphological variables for both PRE and POST groups. DRIL: Disorganization of the retinal inner layers; ELM: external limiting membrane; EZ: ellipsoid zone, RF: retinal fluid.

While VA and CS values were very similar in both groups, colour indexes and some morphological variables showed a slight improvement. In that sense, CRT and PFT decreased in the second visit and the cases with active exudative AMD were reduced from 19 (57.6%) of PRE to only 4 (14.8%) of POST group. In the majority of these cases, retinal fluid showed a subretinal location (47.4% in PRE and 100% in POST). The retinal inner layers were disorganized (DRIL) in 6 PRE (18.2%) and in 3 POST (11.1%) group, while the number of patients with the outer retinal layers altered was greater either in PRE and POST group, especially EZ (Figure1).

Statistical comparison for dependent samples (Wilcoxon signed-rank and McNemar tests) displayed no significant differences ($p>0,05$) between the visual variables of PRE and POST outcomes. This means that visual acuity, contrast sensitivity nor colour vision did not improved after anti-VEGF injection. Related to the morphological results, the variables that had a significant improvement between PRE and POST groups were CRT ($p=0.001$), PFT ($p=0.004$) and the activity pattern of the pathology ($p=0.000$).

Therefore, the next phase of the present study was to analyse if the activity pattern of the disease before anti-VEGF treatment was related to the visual and morphological variables of PRE and POST groups.

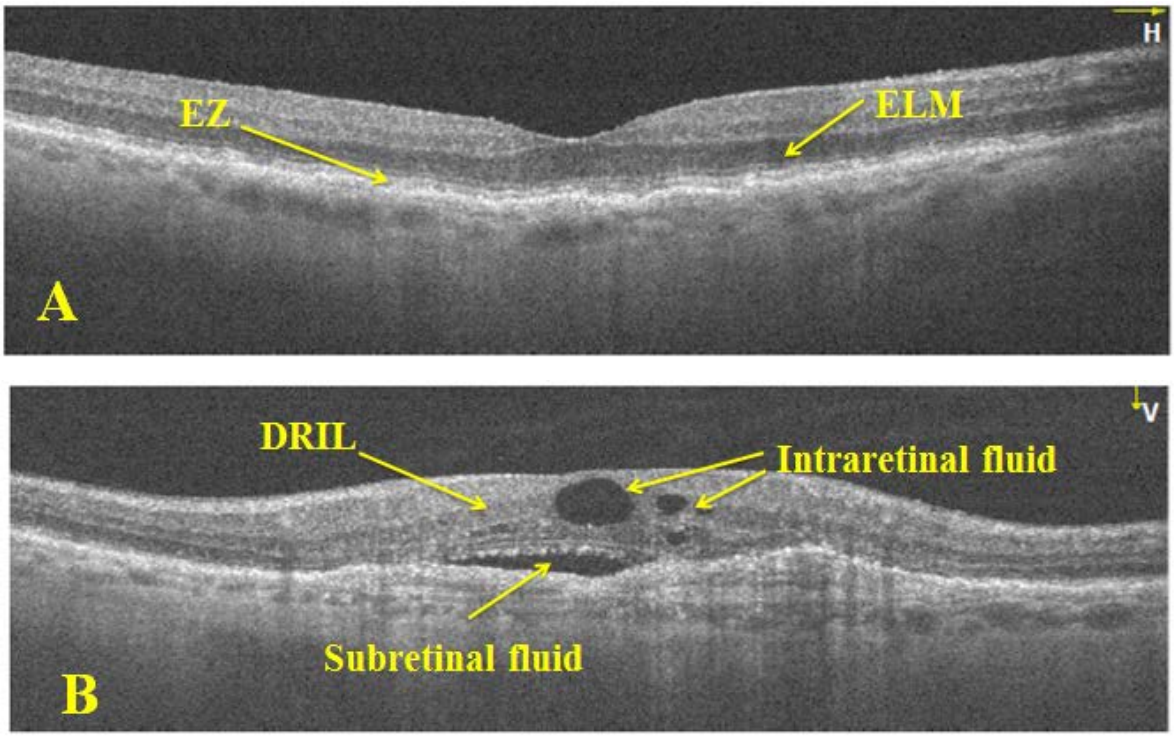

Figure 1. Representative OCT images of two exudative AMD patient. A: retina with relatively preserved morphological traits, and normal central and perifoveal thickness. B: retina with affected morphological traits, and increased central and perifoveal thickness. DRIL: Disorganization of the retinal inner layers; ELM: External limiting membrane; EZ: ellipsoid zone.

2. Non-active vs active exudative AMD outcomes

According to the activity pattern before treatment, the 33 initial patients were distributed into two groups, 14 patients (42.4%) in the non-active group, and 19 patients (57.6%) in the active group. A diagram specifying the different comparisons realised in this phase can be seen in figure 2.

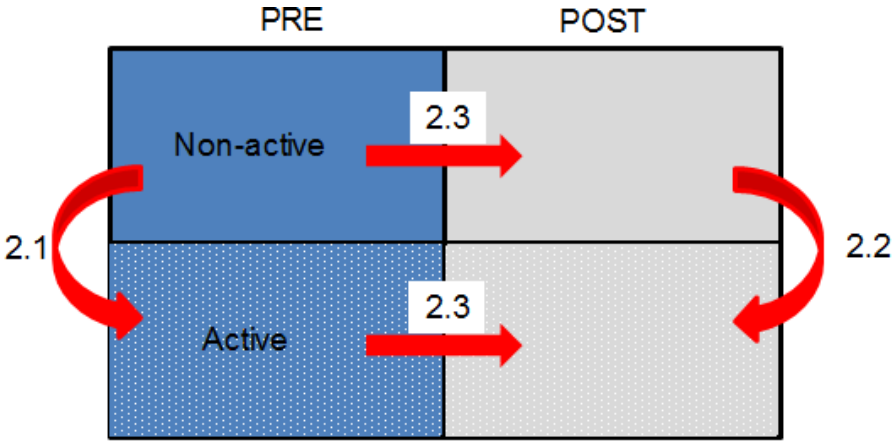

Figure 2. Comparisons between different groups for the second phase of the study.

2.1 Comparison between PRE groups

Summary statistics of visual variables in PRE non-active and PRE active groups are shown in Table 4A (in Supplementary material). In general, non-active retinas displayed better outcomes for VA, CS and color vision indexes than active cases, although no significant differences (p>0,05) were found between both groups.

Descriptive statistics and the frequency distribution of morphological variables are shown in Table 4B and 4C respectively (in Supplementary material). CRT and PFT means were lower in non-active group, including the maximum values. Moreover, outer and inner retinal layers were more preserved in the former group. In that sense, EZ and ELM were apparently normal in 57.1% and 85.7% respectively in non-active cases, in front of 36.8% and 63.2% in active ones, being EZ more altered than ELM, in both groups. Finally, DRIL was only observed in 6 retinas (36.6%) with active exudative AMD (five of them with intraretinal fluid; Figure 3).

The Mann-Whitney U and chi-squared tests for independent samples indicated that, despite values were milder in non-active group, differences were only statistically significant for CRT (U= 71.50; p= 0,025) and DRIL (p= 0.027).

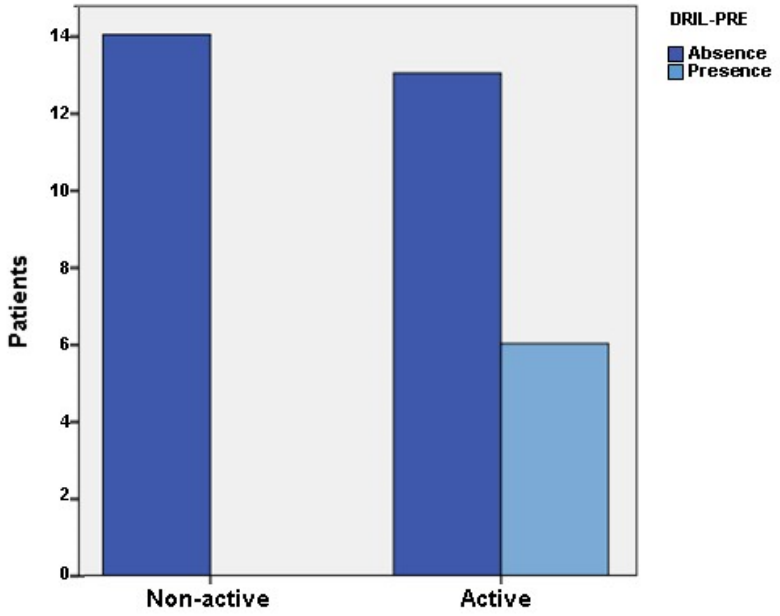

Figure 3. Absence/presence of DRIL according to the disease activity pattern

2.2 Comparison between POST groups

As in the previous section, no statistical differences were found for any of the studied visual variables (Table 5A; in Supplementary material). Regards to morphological variables, retinal thicknesses (Table 5B, in Supplementary material) were lower in the group that showed activity prior to the treatment, while both inner and outer retinal layers were better preserved in non-active group patients (Table 5C in Supplementary material). DRIL only appeared in 3 cases (18.8%) of the active group. Nevertheless, no statistical differences were shown with Mann-Whitney U test for quantitative variables, neither with Fisher exact test for qualitative variables.

2.3 Comparison between PRE and POST groups

To examine if previous activity pattern determined the effectiveness of treatment with anti-VEGF injection (Table 6, in Supplementary material), Wilcoxon signed-rank and McNemar tests for dependent samples were carried out. Despite the increasing number of cases with normal-

appeared retinal layers, no statistical differences were found between PRE and POST outcomes in non-active exudative AMD.

However, in the active cases, CI (p=0.034), CRT and PFT (p=0.001 and p=0.012 respectively; Figure 4) showed statistical improvement after treatment. The resolution of the retinal layer integrity was evident but the small number of cases for any of these variables did not allow the use of any statistical tests. Moreover, the number of retinas that continued active after the treatment was reduced in a drastic manner, remaining only 4 cases with subretinal edema.

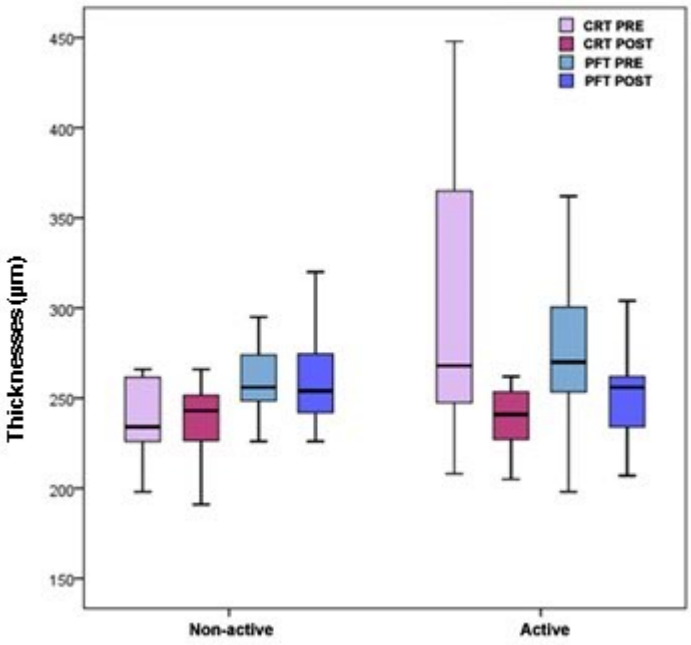

Figure 4. Box-plot of retinal thicknesses outcomes, according to the disease activity pattern

It should be noted that although there were not significant differences between the visual variables of PRE and POST outcomes, these differences were larger in the active cases than in the no-active ones.

3. Comparison of the POST outcomes according to the total number of injections received

Since patients under study had previously undergone various treatments with anti-VEGF intravitreal injections, they were classified in two groups, according to the total number of injections received, regardless of whether they had disease activity or not. Group 1 included patients with at least 4 injections (n=13) and Group 2 patients with more than 4 injections (n=15). In this third phase of the study, only POST outcomes of both groups were compared.

Descriptive statistics and the frequency distributions of visual and morphological variables for both groups are shown in Table 7 (in Supplementary material).When visual variables were compared, the U Mann-Whitney test determined no statistical differences ($p>0.05$) between groups, which means that visual acuity, contrast sensibility and colour vision did not improve after four treatments. With regard to retinal thicknesses, the same test indicated statistical differences in PFT ($p=0.015$; Figure 5) but not for CRT ($p > 0.05$).

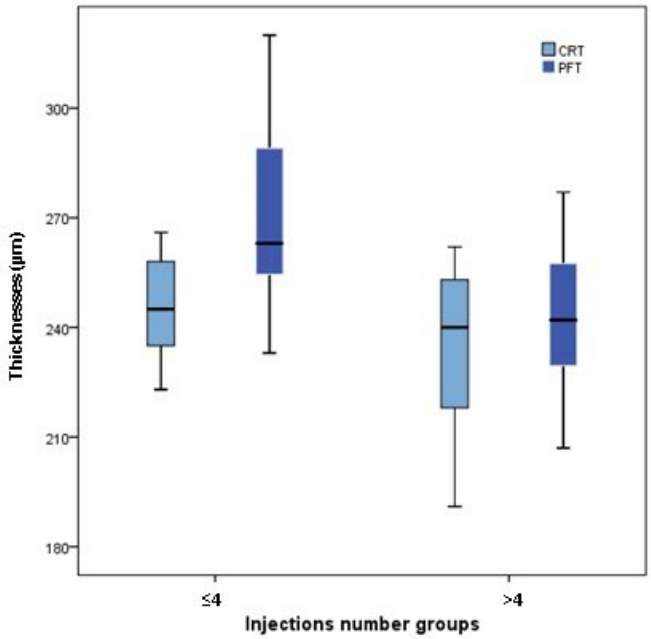

Figure 5. Box-plot of retinal thicknesses outcomes, according to the total number of injections received

In general, the morphological variables were more preserved in Group 1, showing more cases with no altered retinal layers than Group 2 (Table 7C, see annex). Only 3 cases (11.1%) in group

2, showed disorganization of the retinal inner layers, but no case was reported in group 1. While ELM and EZ were only altered in 9.1% of group 1 cases, the percentage increased to 18.8% and 43.8% respectively for group 2. Nonetheless, no statistical differences for any of the morphological variables ($p>0.05$) were found between the two groups, so the number of injections did not seem to have influenced the retinal morphology.

DISCUSSION

The purpose of this study was to evaluate visual and morphological traits of exudative AMD patients from Hospital de Terrassa-Consorci Sanitari de Terrassa (Barcelona, Spain), as well as determine the influence of the activity pattern and total anti-VEGF injections in the effectiveness of treatment.

In the first phase of the present study, the PRE and POST-treatment outcomes were compared. The attained results indicate that one additional anti-VEGF injection had no produced any statistical improvement in visual acuity, contrast sensitivity or colour vision nor in the retinal layer morphology. In contrast, retinal thickness (both CRT and PFT) and the disease activity pattern displayed statistical changes between the first and the second visit.

In fact, these variables have to be related in the sense that the resolution of oedema and, therefore, the loss of the activity by the effect of anti-VEGF injection, involved the reduction of the retinal thickness. It has to be commented, however, that mean CRT value in group 2 POST was lower than values reported after the resolution of oedemas in other maculopathies. [22, 23] Even, it was lower than the 261.31±17.67µm found by Solé et al [24] in 100 healthy eyes. Nevertheless, the same authors indicate than values of retinal thickness depend on the OCT instrument, given 256 µm as a normal value for Optovue OCT device. It would be interesting to determine if the application of repeated injections is the cause of the maintenance of the retinal thickness below the normal values, thus affecting the visual function.

The disease activity between PRE and POST groups decreased noticeably, from 19 to only 4 patients with retinal fluid respectively. Despite this drastic oedema resolution, no substantial improvement in visual or morphological variables was observed.

These results coincide with those reported by Feigl et al [25] in patients diagnosed with age-related maculopathy. The authors showed that VA, CS and colour vision, altered in the first visit study, had not improved one year later. Sabour-Pickett et al [26] also showed no statistical changes in VA between baseline and after one year follow-up, but the mean foveal thickness had diminished significantly from baseline data in patients with exudative AMD. Feigl et al [27] demonstrated that although retinal thickness decreased after 3 anti-VEGF injections in exudative AMD patients, VA and CS remained stable.

Regarding to morphological variables, the presence of intra or subretinal fluid modified the disposition of retinal layers, as will be discussed below.

To sum up, differences between PRE and POST outcomes would suggest the influence of the exudative AMD activity on the visual and morphological traits, and therefore its effect on the treatment effectiveness. In that sense, the analysis of how the disease activity pattern could affect the studied variables indicated that non-active PRE group exhibited better results related to VA and colour vision, as well as more preserved retinal morphology than active PRE retinas. In these active cases, the presence of oedema caused a considerable increase of retinal thickness.

As in the present study, several authors[28-30] indicate that the treatment would produce the reduction of oedema and thus retinal thickness decreased more than in retinas without oedema. In that sense, Barteselli G et al [28] concluded that the resolution of diabetic macular edema induced a significant decrease in the central thickness of the retina. However, unlike what happens in exudative AMD, the edema resolution was not associated with visual function recovery, as it has been proved in other macular pathologies. Yuzbasioglu et al [29] showed that in patients with retinitis pigmentosa treated with anti-VEGF injections, cystoid macular edema disappeared at the same time that central macular thickness decreased and VA improved. Barone et al [30] evaluated patients with pseudophakic cystoid macular edema treated with anti-VEGF injections, and concluded that as the edema resolved, VA increased and central macular thickness decreased significantly.

When comparing PRE and POST outcomes, the improvement in VA and color vision indexes, seems to be better in prior active than in non-active cases, although only CI was significant when

edema existed. In contrast, the morphological variables were more preserved in non-active group. Nevertheless, retinal thicknesses were significant reduced in active disease group, mainly due to the disappearance of retinal oedemas that only remained in four patients with subretinal fluid. Probably, the reduction of oedemas could be also responsible for the greater proportion of retinal layers resolution in this group.

In addition, DRIL appeared mostly in cases with intraretinal fluid, and only in one case with subretinal fluid. After the treatment, the decrease in retinal thickness and the improvement of DRIL and outer retinal layers were better in active PRE group than in no-active PRE one, although in no significant way.

The inability to distinguish boundaries between inner layers (DRIL) on high-resolution OCT imaging may suggest destruction or disorganization of some axons and nuclei of amacrine, bipolar, and/or horizontal cells located in these areas. [22] Pelosini et al [31] and Grewal et al [32] reported that large oedemas can snap bipolar axons and cause loss of visual signaling from photoreceptors to ganglion cells and this fact would explain why the active PRE group presented disorganised retinal inner layers as well as reduced results in visual variables.

In the same way, Radwan et al [23] demonstrated that in patients with diabetic oedema, significant correlation was found between DRIL and oedema, and that oedema resolution involved DRIL disappearance and the visual acuity improvement. Those results are in agreement with the present study, in the sense that only patients with retinal fluid (active group) presented altered retinal inner layers (DRIL) and worse visual and morphological outcomes than those patients with no edemas (non-active group). Likewise, Sun et al [22] also described that DRIL was a stronger marker of VA in patients with diabetic macular edema, showing that extended DRIL implied poorer VA.

Several studies about photoreceptors restoration in different macular pathologies had indicated that EZ and ELM integrity were significantly correlated at all pre and post treatment times.[33] Moreover, the restoration of ELM was early than that of EZ and may reflect morphological and functional recovery of foveal photoreceptors. For that reason, the integrity of the external limiting membrane (ELM) rather than EZ was a better functional indicator.[34] In the current study, there

were always more retinas with affected EZ than ELM layer, corroborating that absence or presence of reflectivity in the EZ layer was not a good predictor for presence of photoreceptors.[35]

In any case, the retina cell destruction may not be completely reversible after resolution of exudative DME activity, and potentially there will be some eyes in which visual function does not recover.[22]

The improvement of the morphological characteristics after the treatment with anti-VEGF agents caused that the number of cases with altered morphological variables, especially in the active group, did not allow the application of comparing statistical tests. Therefore, it would be convenient to repeat this analysis with a larger number of patients.

Finally, in patients with more than 4 intravitreal injections of anti-VEGF agents, neither the visual conditions nor the morphological characteristics would improve with successive treatments. Therefore, after third or fourth injections, the effect of successive injections is basically focused on the reabsorption of the existing retinal oedema and on maintaining the visual and morphological values. In that sense, Ikuno et al [36] and Zhang et al [37] reported that after two and three months (respectively) with anti-VEGF treatment in patients with myopic choroidal exudativeization, treatment generates similar VA outcomes, that is, VA maintenance but no improvement.

In the current study, VA, CS and colour vision were maintained after four anti-VEGF injections. Thus, consecutive treatment seems to be a therapy to stabilize or prevent the progression of pathology rather than to solve the problems caused by the disease. Similarly, no changes were found in morphological retinal variables between both groups. Thus, retinal layers alterations caused by exudative AMD pathogenesis, were not resolved despite the number of injections applied. Therefore, the same as visual variables, the results of the present study suggest that the administration of more than four anti-VEGF injections seems to have a stabilising purpose more than a curative one.

In the same way, Sabour-Pickett et al [26] reported that the greatest visual improvements in patients with exudative AMD treated with intravitreal injections are mainly achieved in the first three months of treatment. Courtney et al [38] also indicated that first three months of anti-VEGF

injections in patients with AMD is a common time point where therapy may be discontinued or treatment interval may be extended, because it is when most important visual and morphological improvements occur. After this early treatment, there is currently no clear consensus regarding the optimal treatment regimen for exudative AMD patients. Recent studies have shown that the TAE regimen results in similar visual acuity outcomes as monthly injections, and that monthly injections and a TAE regimen give better visual acuity than the PRN regimen. [10] Thus, PRN regimen is personalized treatment but it presents the disadvantage that the lesion is retreat when it has already reactivated. In contrast, monthly and TAE protocols are based on retreatment before lesion reactivating but with different number of clinic visits.[5, 6, 10, 11, 13]

As a conclusion, the resolution of oedema and, thus the reduction of retinal thicknesses, seem to be the only substantial improvement that anti-VEGF treatment provides, since neither visual nor morphological changes are statistically significant. However, patients often perceive some visual enhancements after treatment. It could be possible that, although no significant, the small achieved changes in VA, CS and color vision may be important enough to really represent an improvement in the visual perception of these patients. Further studies with a larger population and longer period of follow-up would be of interest to corroborate this new perspective.

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SUPPLEMENTARY MATERIAL

A	NON-ACTIVE eAMD (n=14)				ACTIVE eAMD (n=19)			
	LogMAR	LogCS	TCDS	CI	LogMAR	LogCS	TCDS	CI
Mean	0.28	1.23	213.76	2.35	0.33	1.33	245.05	2.67
Median	0.23	1.20	222.85	2.56	0.34	1.35	246.60	2.81
SD	0.22	0.20	53.10	0.71	0.19	0.18	60.89	0.54
Min	0.00	1.05	122.70	1.13	0.06	1.05	163.90	1.60
Max	0.66	1.65	303.40	3.38	0.70	1.65	406.80	3.54

B	NON-ACTIVE eAMD (n=14)		ACTIVE eAMD (n=19)	
	CRT	PFT	CRT	PFT
Mean	245.29	260.07	295.37	278.53
Median	237.50	253.50	269.00	270.00
SD	30.78	25.09	72.85	41.11
Min	198.00	226.00	208.00	198.00
Max	316.00	322.00	448.00	362.00

C		NON-ACTIVE eAMD (n=14)	ACTIVE eAMD (n=19)
		%	%
EZ	**Normal**	57.1	36.8
	Altered	42.9	63.2
ELM	**Normal**	85.7	63.2
	Altered	14.3	36.8
DRIL	**Absence**	100.0	68.4
	Presence	0.0	31.6

Table 4. PRE-group outcomes, according to the activity pattern prior to treatment. A: descriptive statistics for the studied visual variables; B: descriptive statistics for retinal thicknesses; C: frequency distribution of the studied morphological variables. CI: confusion index; CRT: central retinal thickness; DRIL: Disorganization of the retinal inner layers; ELM: external limiting membrane; EZ: ellipsoid zone; LogCS: contrast sensitivity logarithm; LogMAR: Logarithm of the Minimum Angle of Resolution; PFT: Perifoveal thickness; TCDS: Total Colour Difference Score. Thickness expressed in µm.

A	NON-ACTIVE eAMD (n=12)				ACTIVE eAMD (n=16)			
	LogMAR	LogCS	TCDS	CI	LogMAR	LogCS	TCDS	CI
Mean	0.21	1.32	215.18	2.34	0.27	1.32	220.58	2.43
Median	0.20	1.35	199.40	2.11	0.30	1.35	239.60	2.72
SD	0.187	0.20	71.01	0.90	1.98	0.20	49.10	0.60
Min	-0.06	1.05	117.00	1.00	-0.08	0.90	117.00	1.00
Max	0.64	1.65	341.90	3.67	0.60	1.65	281.50	3.10

B	NON-ACTIVE eAMD (n=12)		ACTIVE eAMD (n=16)	
	CRT	PFT	CRT	PFT
Mean	243.00	261.36	238.93	251.93
Median	243.00	254.00	241.00	256.00
SD	29.53	28.17	30.76	23.93
Min	191.00	226.00	165.00	207.00
Max	309.00	320.00	302.00	304.00

C		NON-ACTIVE eAMD (n=11)	ACTIVE eAMD (n=16)
		%	%
EZ	**Normal**	81.8	81.3
	Altered	18.2	18.7
ELM	**Normal**	90.9	62.5
	Altered	9.1	37.5
DRIL	**Absence**	100.0	81.3
	Presence	0.0	18.7

Table 5. POST-group outcomes, according to the activity pattern prior to treatment. A: descriptive statistics for the studied visual variables; B: descriptive statistics for retinal thicknesses; C: frequency distribution of the studied morphological variables. CI: confusion index; CRT: central retinal thickness; DRIL: Disorganization of the retinal inner layers; ELM: external limiting membrane; EZ: ellipsoid zone; LogCS: contrast sensitivity logarithm; LogMAR: Logarithm of the Minimum Angle of Resolution; PFT: Perifoveal thickness; TCDS: Total Colour Difference Score. Thickness expressed in µm.

A	NON-ACTIVE eAMD		ACTIVE eAMD	
	PRE (n=14)	POST (n=11)	PRE (n=19)	POST (n=16)
LogMAR	0.28 (0.22)	0.21 (0.19)	0.33 (0.19)	0.27 (1.98)
LogCS	1.23 (0.20)	1.32 (0.20)	1.33 (0.18)	1.32 (0.20)
TCDS	213.76 (53.10)	215.18 (71.01)	245.05 (60.89)	220.58 (49.10)
CI	2.35 (0.71)	2.34 (0.90)	2.67 (0.54)	2.43 (0.60)

B	NON-ACTIVE eAMD		ACTIVE eAMD	
	PRE (n=14)	POST (n=11)	PRE (n=19)	POST (n=16)
CRT	245.29 (30.78)	243.00 (29.53)	295.37 (72.85)	238.93 (30.76)
PFT	260.07 (25.09)	261.36 (28.17)	278.53 (41.11)	251.93 (23.93)

C		% NON-ACTIVE eAMD		% ACTIVE eAMD	
		PRE (n=14)	POST (n=11)	PRE (n=19)	POST (n=16)
EZ	**Normal**	57.1	81.8	36.8	62.5
	Altered	42.9	18.2	63.2	37.5
ELM	**Normal**	85.7	90.9	63.2	81.3
	Altered	14.3	9.1	36.8	18.7
DRIL	**Absence**	100.0	100.0	68.4	81.3
	Presence	0.0	0.0	31.6	18.7
ACTIVITY	**No-active**	100.0	100.0	0.0	75.0
	Active	0.0	0.0	100	25.0

Table 6. PRE and POST-group outcomes, according to the activity pattern prior to treatment. A: Mean (SD) for the studied visual variables; B: Mean (SD) for retinal thicknesses; C: frequency distribution of the studied morphological variables. CI: confusion index; CRT: central retinal thickness; DRIL: Disorganization of the retinal inner layers; ELM: external limiting membrane; EZ: ellipsoid zone; LogCS: contrast sensitivity logarithm; LogMAR: Logarithm of the Minimum Angle of Resolution; PFT: Perifoveal thickness; TCDS: Total Colour Difference Score. Thickness expressed in µm.

A	GROUP 1 (n=13)				GROUP 2 (n=15)			
	LogMAR	LogCS	TCDS	CI	LogMAR	LogCS	TCDS	CI
Mean	0.26	1.28	195.25	2.13	0.29	1.32	231.84	2.55
Median	0.18	1.35	169.50	1.96	0.30	1.35	239.60	2.72
SD	0.32	1.90	63.57	0.82	0.17	0.21	47.84	0.57
Min	-0.06	1.05	117.00	1.00	-0.08	0.90	146.80	1.41
Max	1.10	1.65	293.60	3.26	0.60	3.67	341.90	3.67

B	GROUP 1 (n=11)		GROUP 2 (n=15)	
	CRT	PFT	CRT	PFT
Mean	252.64	271.00	231.87	244.87
Median	245.00	263.00	240.00	242.00
SD	28.86	26.15	28.04	19.54
Min	223.00	233.00	165.00	207.00
Max	309.00	320.00	262.00	277.00

C		GROUP 1 (n=11)	GROUP 2 (n=16)
		%	%
ELM	**Normal**	9.91	81.25
	Altered	9.09	18.75
EZ	**Normal**	90,91	56.30
	Altered	9.09	43.80
DRIL	**Absence**	100	81.25
	Presence	-	18.75

Table 7. POST-group outcomes, according to the total number of injections received: GROUP 1:≤ 4 injections GROUP 2: > 4 injections. A: descriptive statistics for the studied visual variables; B: descriptive statistics for retinal thicknesses; C: frequency distribution of the studied morphological variables. CI: confusion index; CRT: central retinal thickness; DRIL: Disorganization of the retinal inner layers; ELM: external limiting membrane; EZ: ellipsoid zone; LogCS: contrast sensitivity logarithm; LogMAR: Logarithm of the Minimum Angle of Resolution; PFT: Perifoveal thickness; TCDS: Total Colour Difference Score. Thickness expressed in µm.

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